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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: §
Donald Morton §
Rishab K. Gupta §
David M. Euhus §
§
Serial No.: 07/431,533 §
§
Filed: November 3, 1989 §
§
For: URINARY TUMOR ASSOCIATED §
ANTIGEN, ANTIGENIC SUB- §
UNITS AND METHODS OF §
DETECTION §

Group Art Unit: 1813

Examiner: C. Dubuvle

Atty Dkt.: CADL.002/PAR

CERTIFICATE OF MAILING
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10/1/93
Date **David L. Parker**

BRIEF ON APPEAL

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I. STATUS OF CLAIMS

The present application was filed as an original application on November 3, 1989, with claims 1-46.

In a restriction requirement set forth in an Action dated October 29, 1991, the Examiner indicated that the claims defined eight distinct inventions. In a response dated December 30,

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1991, the Appellants elected to proceed with the Group I invention, represented by claims 1, 6, 11-19, 39, 40 and 46.

In an Action dated May 18, 1992, the Examiner withdrew claims 39, 40 and 46 from consideration as not readable upon the species elected for prosecution.

In a Response dated October 19, 1992, claims 13 and 18 were amended.

In a Response dated June 3, 1993, claims 1, 6 and 11-17 were canceled, claim 18 amended and claim 47 added. A Notice of Appeal was also filed on June 3, 1993.

Claims 18, 19 and 47 are pending in this Appeal, a copy of which is attached as Exhibit A.

II. STATUS OF AMENDMENTS

Claims 1, 6 and 11-17 were canceled, claim 18 amended and claim 47 added subsequent to the final Action dated December 3, 1992. These amendments were entered by the Examiner. Claims 18, 19 and 47 are pending in this appeal.

III. SUMMARY OF THE INVENTION

The present invention concerns a novel vaccine antigen formulation that includes at least the 100 K subunit of an antigen designated as Urinary Tumor Associated Antigen, or "U-TAA". UTAA vaccine preparations have a variety of intended applications, including use in the preparation of antibodies having specific tumor diagnostic potential (based upon their

tumor selectivity) as well as in vaccination of cancer patients for the development of, for example, active immunity.

Detailed studies are set forth in the specification that demonstrate the preparation and use of UTAA containing vaccines such as "MCV" vaccine (see Exhibit B, an excerpt from pages 16-17, 28-29 of the specification), including details as to how one would proceed to immunize animals with the UTAA vaccine to thereby prepare antibody (see Exhibit C, an excerpt from pages 24-25, 28, 42), and the use of such an antibody in diagnostic assays (see Exhibit D, an excerpt from pages 31-32, 49-51).

IV. ISSUES ON APPEAL

The only issue for the Board's consideration on appeal is whether the Examiner's final rejection of the pending claims on the basis of 35 U.S.C. §112, First Paragraph is appropriate.

V. GROUPING OF CLAIMS

For the purposes of the present appeal, the Board is requested to separately consider the patentability of the pending claims. It is believed that the present Brief is in compliance with 37 C.F.R. § 1.192(c), in that the patentability of the three pending claims is argued separately.

VI. ARGUMENT

A. Rejection of Claims 18 and 19 Under 35 U.S.C. § 112, First Paragraph

Claims 18 and 19 stand rejected under 35 U.S.C. §112, first paragraph, for what appears to be essentially two reasons.

First, the position is taken that the specification does not enable "substantially" pure antigen (set forth in items 17-21 and 26-27 of the Office Action of 12/3/92), and, secondly, that the specification fails to teach the administration of a vaccine composition that inhibits cancer in a patient (items 22-25).

The "Purified" Antigen of Claim 47

In response to the purification issue, Appellants first observe that the issue is moot with respect to new claim 47, in that this claim specifies a vaccine incorporating simply a "purified" U-TAA antigen. There can be no doubt that the specification adequately describes purified U-TAA antigen. Two separate techniques are disclosed, one involving the isolation of U-TAA from urine (see specification, pages 22-23) and the other involving its isolation from serum (pages 32-36). It is demonstrated using these techniques that at least a 100-fold purification is obtained. The Examiner agrees with this position, as evidenced by a statement made in the final Action:

> The specification fails to teach substantially pure subunits of UTAA. <u>While a 100 fold purification is described</u>, it is unclear that this constitutes substantially pure material. (emphasis supplied).

OA of 12/3/92, page 2, paragraph 17.

Claims 47 refers simply to the "purified" antigen, and thus should read upon such antigen that is in any way at least partially purified over naturally occurring antigen.

As an apparently separate basis of rejection, item 27 of the Action posits that Appellants are claiming a purified "subunit". The Action thus takes the position that it must be demonstrated that the 90-100 kD subunit *per se* has been purified away from the holoantigen.

In response, it is not Appellants' intention that claim 47 require that the specified subunit be purified away from the holoantigen -- only that it be purified over nature. Rather, it was intended that claim 47 cover UTAA compositions in vaccine compositions, regardless of whether the 90-100 kD subunit was purified away from the holoantigen. Moreover, the "purified" language was employed in an abundance of caution to distinguish the subject matter of the claim over nature, as required by 35 U.S.C § 101.

It is submitted that Appellants intention that claim 47 also read upon holoantigen is supported by the wording of claim 47. It is implicit in the wording that the 90-100 kD subunit is observed as present in the vaccine after "reduction by β-mercaptoethanol and separation by SDS-polyacrylamide gel electrophoresis". That is, one can observe the subunit as present after reduction and separation of the holoantigen contained in the vaccine.

Accordingly, the Board is respectfully requested to overturn the Examiner's decision that the subject matter of claim 47 is insufficiently enabled by the specification.

The "Substantially Purified" Antigen of Claims 18 and 19

Regarding claim 18 and 19, it is submitted that the term "substantially purified" is appropriate to describe admittedly 100-fold purified antigen and is in no way non-enabled by the specification. As noted above, the PTO apparently agrees that the specification describes and enables a U-TAA antigen preparation that is 100-fold purified over the antigen as it exists in nature (*i.e.*, as it exists in urine), but is apparently concerned with whether such an antigen preparation is "substantially" purified. It is submitted that while "purified" is employed in claim 47 to refer to any degree of purification over nature, the term "substantially purified" is intended to refer to more highly purified preparations, such as those that are 100-fold purified. It is thus respectfully submitted that a substantially purified U-TAA antigen preparation has admittedly been prepared, in that the scope of the designation "substantially" has been defined operationally by the disclosure itself.

As noted above with respect to claim 47, the Examiner appears to take the position that the claims require substantially pure "subunits". While it is believed that the specification enables substantially pure subunits, it is

submitted that the claim was not intended to be limited to the subunit per se, and was drafted to include the holoantigen as well. This is submitted to be clear from the specification, which provides extensive details regarding the purification of UTAA and further characterization and separation of the individual subunits by gel electrophoresis and Western blot analysis.

Appellants' position that the specification is enabling for the claimed substantially purified material is supported by the relevant caselaw. For example, in the case of *In re Doyle*, 140 U.S.P.Q. 421 (C.C.P.A. 1964), the CCPA held that "substantial purification" of a penicillin (6-APA) can distinguish the claim from naturally occurring 6-APA. Furthermore, the use of the term "substantially" has been routinely accepted as sufficiently definite, where, as here, the specification provides an indication of what is intended by the term. See, *e.g.*, *Ex parte Smith*, 43 U.S.P.Q. 157, 158 (PTO Bd. App. 1937).

In that the Action recognizes that Appellants' specification teaches at least relatively purified U-TAA antigen, there must be some means of claiming the invention of claim 18 that would be acceptable to the PTO under 35 U.S.C. § 112, first paragraph.

Vaccine Utility -- Utility of UTAA as an Immunogen for Antibody Preparation and Use

The second basis of rejection under § 112, first paragraph, appears to concern utility and enablement of the claimed vaccine (see paragraphs 22-25 of the final OA). From these statements,

it appears as though the Examiner's concerns are that the specification fails to demonstrate cancer inhibition using the vaccine, and that the evidence is "speculative" (paragraphs 22-24)

It is first submitted that the foregoing concerns are essentially ones of clinical utility and thus not reasonably assertable against the claims. None of the pending claims requires cancer "inhibition" -- all that is required is that the claims define at least one practical utility. See, *Raytheon Co. v. Roper Corp.*, 220 U.S.P.Q. 592 (Fed. Cir. 1983) ("When a properly claimed invention meets at least one stated objective, utility under § 101 is clearly shown." (emphasis supplied)).

In the Advisory Action dated 8/19/93, the Examiner takes the position that vaccine claims "by definition" require that the ability to confer protection be shown. Appellants submit that the Examiner is inappropriately attempting to read a field of use limitation into a product claim. Examiner's are required to interpret a claim as broadly as is reasonable, and cannot attempt to limit product claims to an intended use. The fact of the matter is, if the vaccine has some recognizable utility, whether it be clinical utility or not, then the subject matter of the claim must be accepted as satisfying the utility requirement.

It is submitted that because claims 18 and 47 concern a vaccine *per se*, the utility of these claims is satisfied by any utility, including in the preparation of diagnostic antibodies (see, e.g., specification, page 23, line 7-10; page 28, line 29,

to page 29, line 28; page 36, line 25, to page 37, line 7; and page 41, line 28, to page 44, line 11). Moreover, the *in vitro* diagnostic utility of the subsequent anti-UTAA antibody is demonstrated in the specification. Examples IX (pages 31-32) demonstrates preparation and carrying out of an assay for UTAA. Example XXVIII (pages 49-50) demonstrates the detection of UTAA in the urine of cancer patients -- only 2% false positives with almost 70% known positives identified. Lastly, Example XXIX (pages 50-51) demonstrates a relationship between UTAA and recurrence in the urine of cancer patients.

It is further submitted that claim 19 does not require cancer inhibition in order to satisfy the utility requirement. The claim requires only that the vaccine be employed to induce antibody production, with no mention of cancer inhibition. As such, the claim could just as readily concern a method of antibody production which, as noted above, is a useful endeavor in and of itself.

Thus, in that the claimed subject matter is useful in antibody production, which antibodies are useful in disease detection and prognosis, the subject matter of the claims must be taken as satisfying the utility requirement.

Vaccine Utility - Cancer Inhibition

While it is respectfully submitted that the foregoing adequately satisfies the utility requirement, Applicants bring to the Board's attention the attached 1992 publication they have

authored (Exhibit E) entitled "Prolongation of Survival ...", which demonstrates successful treatment of melanoma using the MCV of the invention. Moreover, this article demonstrates the surprising clinical efficacy of the claimed MCV vaccine in the prolongation of survival of cancer patients. It is submitted that this article clearly demonstrates the clinical efficacy of the MCV vaccine. For example, the enclosed Exhibit E reference, in its abstract, indicates that there has been no change in the prognosis for Stage IIIA and Stage IV melanoma patients in the last 20 years. Yet, in clinical trials, the MCV increased the median and 5-year survival of stage IIIA melanoma two-fold, and stage IV patient three-fold!!

Vaccine Enablement

Lastly, the Action takes the position that Appellants' specification fails to demonstrate immunization with a substantially purified material (item 25 of the Action).

An exemplary vaccine preparation is described, *e.g.*, at page 24 (". . . 100 ug U-TAA mixed with an equal volume of Mylanta (Stuart Pharmaceuticals . ."), and its use in an immunization protocol is described beginning at page 24, line 26 (" . . . four injections of U-TAA over the course of 6 weeks . . .").

Another U-TAA vaccine formulation and its use is described at page 28 (Example V), where vaccines incorporating 75 ug of purified U-TAA in PBS were administered on days 0, 15 and 28 in the preparation of hybridomas.

Lastly, a vaccine formulation is described in Example XX beginning at page 41, with a vaccination protocol given on page 42. Antibody responses and titers are set forth in the subsequent Examples.

Thus, it is evident that the specification discloses clear details regarding the preparation of vaccine formulations. Moreover, actual working examples demonstrate that the vaccine can be employed in the preparation of antibodies that are selectively tumor reactive (see, *e.g.*, Table 5, page 50)

It is respectfully submitted that these portions of the specification more than adequately teach exemplary U-TAA vaccine preparation and use.

V. CONCLUSION

Appellants respectfully submit that from the foregoing observations and arguments, a conclusion of indefiniteness and anticipation of the claims in unwarranted. It is therefore

respectfully requested that the Board overturn the Examiner's rejection.

Respectfully submitted,

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CLAIMS ON APPEAL

18. A vaccine comprising a substantially purified polypeptide subunit of Urinary Tumor Associated Antigen having, after reduction by β-mercaptoethanol and separation by SDS-polyacrylamide gel electrophoresis, a molecular weight of about 90 to 100 kD, and a pharmaceutically acceptable carrier.

19. A method for inducing or enhancing in a subject the production of antibodies reactive with tumor cells in the subject comprising administering the vaccine of claim 18.

47. A vaccine comprising a purified polypeptide subunit of Urinary Tumor Associated Antigen having, after reduction by β-mercaptoethanol and separation by SDS-polyacrylamide gel electrophoresis, a molecular weight of about 90 to 100 kD, and a pharmaceutically acceptable carrier.

as well as during and after treatment. Initially, U-TAA levels may be very high indicating a high turnover or shedding of the antigen. However, after treatment and inhibition of proliferation of tumor cells by vaccination, for example, U-TAA levels in a patient's body fluids may decrease.

The invention allows one to diagnose a tumor in a subject by the method comprising detecting the epitope located on the 45, 65, 90-100, or 120 kD polypeptide from U-TAA in a subject's body fluid after reduction by ß-mercaptoethanol and separation by SDS-polyacrylamide gel electrophoresis. The detection can be accomplished by contacting the polypeptide with a reagent and detecting the presence of the reagent which is reactive with the polypeptide.

The invention describes a method of detecting a breast or lung carcinoma in a subject comprising detecting the presence of U-TAA from a sample of the subject. The detection comprises binding the U-TAA with a reagent and detecting the reagent. One example of detection is the binding of U-TAA directly or indirectly by a second reagent. The reagent is preferably an antibody but can be any suitable reagent.

A vaccine is provided for inducing or enhancing antibodies or cell mediated immunity directed against the 90-100 kD polypeptide comprising tumor cells having a U-TAA on the cell surface and at least one tumor associated antigen selected from the group consisting of GM-2, GD-2, Fetal Antigen, or Melanoma-Tumor Associated Antigen, and a pharmaceutically acceptable carrier. Improved results are possible if the tumor cells have HLA of the same type as that of the subject on the cell surface. The vaccine provides a method for inducing or enhancing in a subject afflicted with a cancer the production of antibodies

reactive with the polypeptide subunit of U-TAA having a molecular weight of about 90 to 100 kD, comprising administering to the subject an effective dose of the vaccine. The subject of the present invention is a human being, however, any animal may be used. The antibody produced in the individual after administration of the vaccine inhibits or treats the cancer, for example a melanoma. Inhibiting the cancer refers to the ability to contact the tumor cells with a reagent which can prevent the cells from proliferating, thus resulting in cell death and a reduction in size of the tumor. Alternatively, inhibiting can include a direct cytotoxic effect on the tumor cells.

In addition, the invention provides for development of reagents which are reactive with antibodies which are reactive with Urinary Tumor Associated Antigen. These reagents can be anti-idiotype antibodies which refer to immunoglobulins which bear the internal image of the antigen of interest. Idiotypes are antigenic determinants of the antibody combining site, therefore, anti-idiotype antibodies mimic the antigenic epitope of an antigen. The invention provides a method of immunotherapy comprising injecting into a subject a therapeutic amount of the anti-idiotypic antibody. The therapeutic amount is any amount effective to produce a cytostatic or cytotoxic effect on the tumor cells which can readily be determined by one skilled in the art.

The discovery that U-TAA is found on the surface of tumor cells allows a method of treating a tumor expressing U-TAA on the tumor cell surface in a subject comprising injecting into the subject a tumor inhibiting reagent reactive with U-TAA on the tumor cell surface. The reagent may be an antibody and the antibody may be attached to a cytotoxic or cytostatic agent. The cytotoxic or cytostatic agent, for example may be selected from the group

EXAMPLE V

Murine Hybridoma Technique: An eight week old brown female C57BL/6 mouse was injected intraperitoneally (ip) with 75 μg of U-TAA (Ne8704) in PBS on days 0, 15, and 28. On day 37, the mouse was boosted with 150 μg U-TAA ip and sacrificed three days later to obtain hyperimmune spleen cells. The cells were fused with 8-azaguanine resistent, non-secreting mouse Sp 2/0 myeloma cells in a manner similar to that described by Galfre (Galfre, G. et al., Nature 277:131 (1979) incorporated by reference herein). Hybridoma cells were seeded onto plates containing 3 day old mouse peritoneal macrophage cultures obtained by rinsing the peritoneum of a C57BL/6 mouse with 5 ml of 11.6% sucrose and plating the macrophages at 1.45×10^4 cells/well.

Supernatants from wells containing healthy colonies were screened for anti-U-TAA antibody in ELISA. Positive wells were cloned using the method of limiting dilutions. Monoclonal antibody was prepared from mouse ascites (Hoogenraad, N. et al., J. Immunol. Methods 61:317-320 (1983) incorporated by reference herein). Antibody isotypes were determined by double immunodiffusion (Miles Laboratories, Naperville, IL).

EXAMPLE VI

Target Antigens for Hybridoma Supernate Screenings: Hybridoma supernatants were tested in ELISA for reactivity with various U-TAA preparations and normal urines. U-TAA was purified from urine samples of three different melanoma patients, Ne8704, Wo7907, and Se8703, by Sephacryl S-200 gel filtration chromatography. Ten normal urines were used as target antigen after 100-fold concentration. Each target was used at 1.4 μg protein/ml. In each case antigen was diluted in 0.06 M sodium carbonate buffer (pH 9.6) and

immobilized on microtiter plates by incubation for 3 hr at 37°C. This and each subsequent incubation was followed by three washes in PBS supplemented with 0.05% Tween-20 (PBS-T). Alkaline phosphatase conjugated to goat anti-mouse Ig (Jackson Immunoresearch, West Grove, PA) provided the catalyst for conversion of the non-chromogen, P-nitrophenyl phosphate (1 mg/ml in 10% diethanolamine, pH 9.6), to chromogen, P-nitrophenyl. The absorbance of each well was measured at 405 nm using the multiscan ELISA plate reader.

During the course of immunization the anti-U-TAA antibody activity in the mouse serum increased from undetectable to 1:8000 against Ne8704 and 1:7730 against Wo7907 by ELISA. All of the 96 wells seeded with fusion products of the immunized mouse spleen cells grew healthy hybridomas. Supernatants of 51 of these hybrids contained antibody reactive with Ne8704, but only 13 were positive against both Ne8704 and Wo7907. Cells from these 13 wells were cloned. Among the resultant clones, only one designated as AD1-40F4 produced an antibody that reacted with U-TAA of Ne8704, Wo7907, and Se8703 urine samples but not with two concentrated normal urines. AD1-40F4 proved to be an IgM molecule by double immunodiffusion. Unprocessed hybridoma supernatant of this clone had an anti-U-TAA IgM titer of 1:200. AD1-40F4 raised in mouse ascites had an anti-U-TAA IgM titer of 1:2000-1:5000 and was used in subsequent experiments at 1:200-1:500 dilutions.

EXAMPLE VII

Specificity Analysis of AD1-40F4: A murine monoclonal antibody capable of differentiating between U-TAA and normal urine was raised in mouse ascites and tested for reactivity with a variety of immobilized targets (Euhus, D. et al.,J. Clin. Lab. Anal. 3:184 (1989)). The ascite was used at 2 μg/ml in all of the following assays. To assess

passed through a 35 ml bed volume of DEAE-Affigel Blue and eluted with the potassium phosphate buffer. The eluent was collected in 10 ml fractions. The fractions demonstrating absorbance of greater than 0.05 at 280 nm were pooled and concentrated to 10 ml. The allogeneic anti-U-TAA IgG level of the concentrate was measured by an enzyme linked-immunosorbent assay (ELISA). The IgG preparation contained 2.23 mg protein/ml and had an anti-U-TAA titer of 1:2560.

Proteins that remained bound to the DEAE Affigel Blue were eluted with 0.02 M phosphate buffer supplemented with 0.5 M NaCl (pH 8.0) and concentrated to 10 ml. The concentrate was subjected to Sephacryl S-300 (Pharmacia Uppsala, Sweden) gel filtration chromatography to enrich anti-U-TAA IgM antibody. The fractions containing anti-U-TAA IgM antibody activity by ELISA were pooled and concentrated. The IgM preparation contained 0.89 mg protein/ml and had an anti-U-TAA titer of 1:150. These antibodies have been used successfully in a capture assay for U-TAA (Euhus, D., et al., The FASEB Journal 2(6):A1836 (1988)). While the isolated anti-U-TAA IgM and IgG antibodies reacted strongly with the U-TAA, they showed minimal or no reactivity with normal urine components.

EXAMPLE III

Production of Xenogeneic Anti-U-TAA Serum: Anti-U-TAA xenoantisera were prepared in the baboon and were used in ELISA and immunoblotting for the detection of U-TAA. A 12 year old male baboon weighing 28 kg was injected intramuscularly at predetermined (one to four week) intervals with 100 μg U-TAA mixed with an equal volume of Mylanta (Stuart Pharmaceuticals, Wilmington, DE). The baboon was bled periodically and serum anti-U-TAA levels were measured by ELISA.

After four injections of U-TAA over the course of 6

weeks, the baboon developed detectable anti-U-TAA IgG levels. The antibody titers peaked at the 40th week. At this time the anti-U-TAA antibody titer by ELISA was 1:200,000.

IgG and IgM antibodies from the baboon serum were purified by DEAE Affi-Gel Blue chromatography as described above in Example II (Figure 2). IgG antibody was used in the double determinant EIA and IgM for cytotoxicity studies.

EXAMPLE IV

Cytotoxic Effect of Baboon Anti-U-TAA Antibodies: Both IgG and IgM baboon anti-U-TAA antibodies were tested for their cytotoxic effect in the complement dependent cytolysis (CDC) assay using UCLA-SO-M14 as the target. Although the baboon anti-U-TAA IgG antibody was not cytotoxic under the experimental conditions described below, the IgM antibody was cytotoxic.

Target cells (tumor and control) were harvested in mid log phase of growth, washed twice with RPMI-10% FCS, and labeled with 51 Cr. The labeled cells were seeded into the cytotoxicity assay plate at a concentration of 1.0×10^4 cells/well in 50 μl volume. The cells were then mixed with 50 μl of baboon IgM antibody (30 μg protein/ml) and incubated at 4°C for 1.0 hr, followed by the addition of 100 μl baby rabbit complement at 1:10 dilution. The assay plates were further incubated at 37°C for 2 hrs. After centrifugation of the plates (500 X g for 5 min), 100 μl supernates from each well were aspirated and radioactivity release was assessed by gamma counting. Maximum lysis (total radioactivity added to each well) was determined by adding 150 μl of 0.05% Nonadet P-40 (NP-40) in PBS. The spontaneous release of the isotope was determined from the supernate of those wells that did not receive the antibody.

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antibody response to cell membrane antigens, patients were chosen on the basis of their antibody response to M-TAA; eight of the 15 patients had demonstrated induction of anti-M-TAA antibodies during the course of vaccination, while 7 did not. This also allowed us to determine whether antibody response to one membrane antigen guaranteed antibody response to other antigens on the same cell surface.

Vaccination Protocol: First vaccination comprised of 24 million live, irradiated tumor cells (from cell lines designated UCLA-SO-M10, UCLA-SO-M24 and UCLA-SO-M101 which were selected for their antigenic variability) mixed with 24 million Glaxo BCG organisms. Second vaccination consisted of a mixture of 24 million live, irradiated tumor cells and 12 million Glaxo BCG organisms. Subsequent vaccinations consisted of 24 million live and irradiated tumor cells without BCG. The immunization schedule consisted of biweekly vaccinations for the first six weeks and every four weeks thereafter.

Statistical Analysis: Antibody titers in various groups are expressed as means ± standard error of the mean (SEM). Relevant comparisons were made using students' paired T-tests and Chisquare analysis. The statistical software employed in these analyses was StatView (Brain Power, Inc., Calabasas, CA) for the Macintosh computer.

EXAMPLE XXI

Determination of Serum Anti-U-TAA Antibody Titers: Forty nanograms of U-TAA in 100 µl of 0.06M carbonate buffer (pH 9.6) was dispensed into 96-well polystyrene microtiter plates. Following a 3 hour incubation at 37°C, the wells were washed 3 times in PBS-T, and 100 µl of serially diluted serum was applied. After a 45 minute incubation at 37°C, the wells were washed and anti-U-TAA

grows well in chemically defined medium. Concentrated spent media fractionated on a sepharose 6B column was tested in ELISA as the target for murine monoclonal antibody AD1-40F4. The material in the void volume of the column reacted at a dilution of 1:23,000 with AD1-40F4. This dilution corresponded to a protein concentration of 177 ng/ml.

EXAMPLE VIII

ELISA: Target antigen or capturing antibody was diluted in 0.06 M sodium carbonate buffer (pH 9.6) and bound to polystyrene microtiter plates (Immulon I, Dynatech Laboratories, Inc., Alexandria, VA) by incubation at 37° C for 2 hr in 100 µl/well aliquotes. The plates were then washed three times with PBS supplemented with 0.05% Tween-20 (PBS-T). Each subsequent reagent was diluted in PBS-T and added in 100 µl aliquotes per well. Each reagent addition was followed by a 45 minute incubation at 37°C and three PBS-T washes. As the final step, 200 µl of para-nitrophenyl phosphate at 1 mg/ml in 10% diethanolamine (pH 9.8) as substrate for the enzyme was added to each well. The plates were incubated at 23°C and color development in each well was measured as absorbance at 405 nm using a Multiscan EIA plate reader (Flow Laboratories, Inc., McClean, VA). Each assay was run in quadruplicate with positive and negative controls, as well as controls for non-specific protein binding and conjugate binding to the immobilized antigen or antibody.

EXAMPLE IX

Double Determinant EIA: Initially, the U-TAA content of fractionated melanoma urine was measured using a double determinant EIA, which employed allogeneic anti-U-TAA IgM and IgG antibodies from melanoma patients. In most subsequent experiments, U-TAA content was measured in a

xenogeneic double determinant EIA employing murine monoclonal antibody AD1-4OF4 and baboon polyclonal anti-U-TAA IgG.

In the allogeneic antibody assay, microtiter plates were sensitized with IgM (allogeneic) antibody at 20 μg/ml. Serially diluted standards and urine fractions diluted 1:10 in PBS-T were then applied. Allogeneic IgG antibody at 20 μg/ml in PBS-T was added as the second antibody. Alkaline phosphatase conjugated to goat anti-human IgG (Sigma Chemical Co., St. Louis, MO) diluted 1:1000 in PBS-T was used as the enzyme conjugate. The absorbance at 405 nm was recorded after a 2 hr incubation with the substrate. The U-TAA concentration of each sample was obtained by interpolation from the standard curve. The assay has a sensitivity of 570 ng/ml.

In the xenogeneic double determinant EIA murine monoclonal antibody AD1-4OF4 at 176.0 μg/ml was used to capture the antigen, while baboon IgG at 8.9 μg/ml was used as the second antibody. This assay has a sensitivity of 50 ng/ml.

EXAMPLE X

Sera for U-TAA Activity: Blood was collected by venipuncture from 52 melanoma patients with disease metastatic to regional lymph nodes, in transit lymphatics, or distant visceral tissues and allowed to clot at room temperature for 2 hrs. Sera was separated from the clotted blood by centrifugation at 800 x g for 15 minutes and immediately frozen at -35°C. Sera from 20 apparently healthy controls was obtained and processed in a similar manner. On the day of assay, the serum samples were thawed, diluted 1:10 in 0.06 M carbonate buffer, and applied to microtiter plates in 100 μl aliquotes and incubated at 37°C for 2 hrs. After washing, the wells were

produce IgG responses. Median time to response was 14 weeks. The responses in these 6 patients lasted from 4 to > 40 weeks, with a median of > 10 weeks.

EXAMPLE XXVII

Stage of Disease and Prior Anti-M-TAA Response: Of the 15 patients evaluated in this study, 11 had disease confined to the lymph nodes or locoregional subcutaneous tissues, while 4 had distant metastases. All 4 patients with visceral metastases (3 with pulmonary metastases and 1 with a liver metastasis) mounted good anti-U-TAA IgG responses. Prior to vaccination, these anti-U-TAA IgG levels ranged from 1:1149 to 1:2816 (mean 1:2184 ± 362). Postvaccination the titers peaked at 1:5000 to 1:14,848 with a mean of 1:8777 ± 2117, a 4-fold increase for the group.

Anti-melanoma-TAA antibody levels from sequential serum samples were available for all 15 patients evaluated in this study (Gupta, R.K. et al., Proc. Amer. Soc. Clin. Oncol. 6:249 (1987) incorporated by reference herein). Eight of these 15 patients had mounted good anti-M-TAA antibody responses with MCV. Augmentation of anti-melanoma-TAA antibody levels did not correlate with augmentation of anti-U-TAA IgG levels (R^2=0.279). Overall, the anti-U-TAA response rate (IgM and/or IgG) is higher for M-TAA responders than for M-TAA non-responders, but the difference is not significantly different (X^2, P >0.3).

EXAMPLE XXVIII

Detection of U-TAA in the Urine of Patients with Breast, Colon and Lung Carcinomas: Urine samples obtained from patients suffering from carcinoma of the breast, colon

or lung were analyzed for the presence of U-TAA using the double determinant EIA that employed murine monoclonal AD1-4OF4 and baboon polyclonal anti-U-TAA IgG as described in Example IX. The incidence of the presence of detectable levels of U-TAA is listed in Table 5. These incidences are quite comparable with that of melanoma patients (63.4%).

Table 5

Incidence of U-TAA in urine of patients with carcinoma of the breast, colon and lung by the double determinant EIA.

Histologic Type	# Tested	# Positive	% Positive
Breast Carcinoma	14	9	64.3
Colon Carcinoma	13	9	69.2
Lung Carcinoma	7	3	42.8
Sarcoma	17	10	58.8
Normal	77	2	2.6
Melanoma	115	73	63.4

*Murine monoclonal antibody AD1-4OF4 was used to capture the antigen and baboon polyclonal antibody to detect the captured antigen as described in Example IX. An EIA value of greater than 0.68 was considered positive.

EXAMPLE XXIX

Monitoring of Malignancy Using U-TAA Levels in Urine of Cancer Patients: Urine samples collected sequentially in a prospective manner from 31 melanoma patients treated by curative surgery were analyzed for U-TAA level by the double determinant EIA as described in Example IX with the following modification. The samples were heated at 100°C in a boiling water bath for 2.5 min, cooled in an ice water

bath for 5 min. and mixed with equal volume (200 µl) of 0.025M phosphate buffered saline supplement with 0.5% Tween-20. One hundred microliters of these mixtures were tested in the capture assay. Of the 31 patients, the U-TAA ELISA value of 10 patients remained negative (<0.68 OD_{405}) during the course of their monitoring for one year. Of these, only one (10%) has thus far had clinically detectable recurrence of the disease (Table 6). Recurrence in this patient occurred 24 weeks after entering the study. In contrast, the urine samples of 21 of 31 patients became U-TAA positive (>0.6 OD_{405}). Of these U-TAA positive patients, 12 (57%) have developed clinically detectable recurrence within 0 to 24 weeks). In view of the U-TAA positive results, this group of patients can be considered at high risk of recurrence. Analysis of sequential urine samples of patients with other malignancy can be used to monitor subclinical recurrence of the disease.

Table 6

Relationship between detection of U-TAA in urine of melanoma patients and recurrence of clinically detectable disease in a prospective study of one year.

U-TAA	# of Patients with recurrence	# of Patients without recurrence	Total
Negative	1	9	10
Positive	12	9*	21
		TOTAL	31

p <0.05 by students t-test

* patients with high risk of recurrence.

EXAMPLE XXX